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Some Actions of Sodium Salicylate on Scorbutic Guinea Pigs

Jacob John Giardina
Loyola University Chicago

LOYOLA UNIVERSITY

SOME ACTIONS OF SODIUM SALICYLATE ON SCORBUTIC GUINEA PIGS

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JACOB JOHN GIARDINA
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Vitae

I, Jacob John Giardina, was born May 20, 1910 in Steubenville, Ohio of parents of italian extraction. I have lived in Chicago since 1915 and received my elementary education in the parochial and public schools of this city.

In the fall of 1928, I attended Loyola University, College of Arts and Sciences, receiving the degree of Bachelor of Science in June of 1932. The following September I entered the School of Medicine of the same institution and after completing two years of study, was awarded a fellowship in the department of Physiology and Pharmacology for the term 1935 to 1936 during which this research was done.

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OBJECT

This research was suggested by a recent publication of Rhinehart and Mettier (10) in 1933 in which they infer that rheumatic fever may be founded upon a condition of Vitamin C deficiency and superimposed infection. It was the purpose of this paper to question the action of sodium salicylate upon guinea pigs which were subjected to the condition of experimental scorbutus in order to determine what if any action this substance may have on the scorbutic state since it seems to have such a widespread use in the treatment of rheumatic fever.

The procedure used was to administer sodium salicylate in the diet with and without vitamin C and to observe the growth weight of the animals and also the gross pathological tissue changes that developed.

Introduction

Rhinehart and Mettier (9, 10, 11) have related experimental scurvy plus a superimposed infection to rheumatic fever. They point out as evidence the similarity of the fundamental pathology of the two conditions, namely, degenerative changes in the intercellular tissues. They claim therefore that a vitamin C deficiency background may be necessary for the development of rheumatic fever, when the insult of infection is combined with the scorbutic state.

Wolbach and Howe (13) also characterize the condition of scorbutus as the inability of the supporting tissues to produce and maintain intercellular substance, advancing the theory that scorbutus is the result of the absence of an agent common to all supporting tissue and which is responsible for the setting or jelling of a liquid product. Ascorbic acid (crystalline vitamin C) was found to induce reparative changes in scorbutus by Menkin, Wolbach, and Menkin (6) who furnish additional evidence that this substance is indistinguishable from vitamin C. They discuss the possible mechanism involved in this reaction from the standpoint of the properties of ascorbic acid as a reducing agent in relation to cellular oxidations. Purr (8) furnishes further proof that ascorbic acid is not limited to its antiscorbutic action but has a more compre-

hensive role and that by its reversible oxidation and reduction it is indirectly related to cell respiration and to the activation of the enzyme arginase which is important in the metabolism of proteins.

It may reasonably be assumed that a superimposed infection would in the scorbutic state affect the weakest structure, therein attacking the intercellular cementing mechanism in the orgainism. Gross, Loewe, and Eliasoph (5) met with failure in attempting to reproduce rheumatic fever in seven species of animals by innoculation of streptococci taken from proven rheumatic patients. This holds true for all work presented thus far in the literature with the exception of Rhinehart and Mettier who in a later paper (11) found that neither scurvy nor infection alone can produce the characteristic lesion of rheumatic fever, but that when conbined, the typical lesion occurs with considerable frequency. They find that there is a definite tendency for arthropathies in the scorbutic animal with all the symptoms of joint pains, swelling and hemorrhage and with a definitely increased tendency for arthropathies in scurvy animals subjected to superimposed infection.

The use of salicylates in rheumatic fever was suggestive of another means of testing this hypothesis of its origin on the basis of a preceding scorbutic state. It is known that salicylates have no effect on the primary lesion of rheumatic fever but that it does give relief of symptoms caused by secondary involvements of the joints. Since it has such a

widespread use in the latter condition, it suggested a possible action of salicylates in scorbutus if this substance were effective. Thus a study of the growth weight curve of guinea pigs on a salicylate diet with and with out vitamin C was carried out to see if such effects existed.

Guinea pigs were chosen as experimental animals because they are highly susceptible to scurvy and develope the disease within a relatively short time. Their ability to store and retain Vitamin C is very much less than any other laboratory animal. Their vitamin C requirement is the same as that of the monkey and about five to ten times greater that the human being per unit of weight according to Barnes and Hume (2). They develope symptoms of scurvy in two to three weeks in comparison to eight to ten weeks for the monkey and six months for the human being. The number of animals available for the experiments was also a factor.

Rats and rabbits are not favorable animals for vitamin C defieiency experiments because the former does not develope scurvy on deficiency diets because they seem able to manufacture their own antiscorbutic substance. Drummond (4) has shown that rats existing on a scorbutic diet do not thrive so well and are not so fertile, yet Parson and Hutton (7) have demonstrated that the liver of rats contain appreciable amounts of antiscorbutic substance after they were kept on scorbutic diets for long periods of time, this was true also of the

second generation kept on the same diet. Findlay (3) found that rabbits, although they lose weight and die on vitamin C deficiency diets, do not develope the characteristic symptoms of scurvy.

Briefly, the symptoms of scurvy in guinea pigs are tenderness and swelling of the joints which is one of the earliest signs. The animal is sometimes found lying on its side and supports the affected member in the air or squats in a peculiar hunched-up position in its cage. When the condition of the gums and jaw is severe the animal will assume a 'scurvy face-ache position' with the side of the face lying on the floor. When active the animal hops around with a characteristic stiffness of the hind legs which it has difficulty in extending due to the tenderness and swelling. When the teeth become loosened and the gums and jaws tender, the animal may refuse solid food. In the post-mortem findings, the gross lesions closely resemble those found in the human disease. Hemorrhages are seen anywhere in the body, but are most frequent in the limbs. The ribs are swollen and often fracture at the juncture of bone with cartilage while hemorrhage is relatively frequent. Hemorrhage into the adrenal gland is one of the earliest findings.

Method

Guinea pigs of mixed sexes and varying in weight from 200 to 400 grams were obtained from the Loyola colony. All the animals were placed on the basal diet of Sherman, LaMer, and Campbell (12) which consisted of dried oats 59 percent, dried milk 30 percent, butter 9 percent, cod liver oil 1 percent and salt 1 percent. Vitamin C was supplied by cevitamic acid, 2 milligrams per day, or in tomato juice in excess of 4 cubic centimeters per day for each animal. The weight of each animal was recorded twice weekly throughout the entire experiment.

Basal diet and vitamin C was given to every animal for a period of at least two weeks and the growth weight curve carefully checked for any irregular fluctuations which were considered undesirable. Such fluctuations which were deemed irregular were sudden gain and losses of a degree which would render interpretation of a weight curve difficult, or inconsistent rate of gain which indicated lack of adaptation of the guinea pig to the diet used in this experiment. About 60 animals were started but due to irregulatities in gain of weight only 45 animals were qualified to continue the experiment after this preliminary two weeks period. They were then placed on a basal diet without tomato juice until no further gain in

weight took place. Within the next five days after this peak weight was reached the animals began to lose weight, and when this reached an amount between 10 and 30 grams, it was taken as a definite indication of the onset of a scorbutic state in the animal. It was found that once a loss in weight of this amount occurred it progressed until the death of the animal unless proper treatment with vitamin C was given. At the onset of the scorbutic state as determined in the above manner the diet was then changed to one of the following:

I. Basal diet plus tomato juice or cevitamic acid.

II. Basal diet plus tomato juice and sodium salicylate (1%) incorporated in the diet.

III. Basal diet plus tomato juice and sodium salicylate (1½%) incorporated in the diet.

IV. Basal diet only.

V. Basal diet plus sodium salicylate, (1%).

VI. Basal diet plus sodium salicylate (1½%).

Groups I, II, III, and IV are control groups. The first, to determine the recovery weight curve in treated scurvy; the second and third, the effected of sodium salicylate on the recovery weight curve in treated scurvy; and the fourth, to determine the typical scorbutic weight curve and the time of death. Groups V and VI include the animals used to determine the effects of salicylate on the scorbutic state. The survival period and death weight of each animal was carefully noted

and post-mortem examination for the gross lesions of scurvy was made. The results have all been recorded upon graph paper as growth weight curves. Animals have been grouped according to experimental diet.

Results

In group I, basal diet with vitamin C, the results showed a complete recovery from scurvy evicenced by continuous rise in the growth weight curve. Group II animals, receiving in addition to vitamin C, 1 percent sodium salicylate in the diet also showed a continuation of growth; similarly those in group III with a concentration of 1½ percent sodium salicylate with vitamin C showed a progressive gain in weight. Animals on basal diet only, group IV, after reaching their peak weight showed a progressive loss in weight which finally ended in death after an average survival period of 17.2 days. They retained about 55 percent of their peak weight at death. Group V and VI receiving 1 percent and 1½ percent of sodium salicylate, respectively, in the diet simulated the results observed in group IV, a progressive loss in weight which also ended in death. Those receiving 1 percent sodium salicylate lived an average of 20 days after reaching their peak weight and retained about 62 percent of their weight, while those on 1½ percent diet had a survival period of 15.7 days with 64 percent of their peak weight at death.

Individual animals, placed on the basal diet following the preliminary period, were observed to require different

lengths of time for the development of scurvy as indicated by a beginning loss in weight. Some of them exhibited a loss after 5 days while others were carried as long as 22 days. Tables I to VII, columns 1 and 2, contain the time interval and the amount of gain before the weight loss started.

It was evident after the first few groups were put through the procedure, that discrepencies in the amount of loss after the peak weight was reached, and before the change to the experimental diet, was of great importance. A loss of 20 grams in a guinea pig of 200 grams would be equivalent to 10 percent of his weight, while the same amount in a guinea pig of 400 grams weight would equal only 5 percent of his weight. A fluctuation of 5 percent could be expected in a normal animal. A result expressed in actual grams could easily be misinterpreted as an indication of scurvy or as due to some experimental procedure unless all animal weights were identical. Graph V will bring out this point. It will be noted that each large division represents 25 grams. The curve is that of a group of animals which weighed approximately 425 grams. The weight loss before the change in diet on the 22nd. day was about 15 grams or 3 percent of the total weight. Note the subsequent rise inweight after the change in diet and also the characteristics of the curve when it descends again. It is very similar to that of the animals recorded on graph VI in which the average weight was only about 225 grams

and the weight loss after the peak was reached amounted to almost 10 percent. This group did not show a rise as the former did although both were on the same diet. It was concluded that a percentage loss of the total weight should be sustained before an animal was placed upon a diet of experimental procedure. This percentage loss of weight was arbitrarily taken as 10 percent.

Further analysis of the graphs on a quantitative basis required the use of a common figure for the discussion of the weight of animals since these weights varied from 200 to 400 grams. The percentage weight was calculated so that the peak weight represented 100 percent and all other weights of an animal were calculated as a percent of this peak weight. The peak weight was chosen to represent 100 percent because in the scorbutic group as with the scurvy animals treated with salicylated, it was the highest weight reached and also the turning point which indicated the onset of scurvy. All calculations considered significant have been entered in Tables I to VII.

Table I reveals that the animals on basal diet with vitamin C gained during the initial diet period on basal only. They showed an average gain of 18.6 percent in 11.5 days. They required an average of 3.7 days to lose 10.2 percent of their peak weight. At the end of 32 days after the peak was reached, which was taken as an arbitrary end of the experi-

mental period for the recovery animals, they showed a gain in weight of 114 percent of the peak weight. The animals on basal diet with vitamin C and 1 percent salicylate, Table II, showed a slight gain in weight during the initial period and required 6.6 days to lose 7.6 percent of the peak weight before the diet was changed. At the end of 32 days of the salicylate diet they showed a recovery weight of 134.6 percent of their peak weight. This is 20.6 percent above the animals of the first group. Table III shows that the animals on basal diet with vitamin C and 1½ percent salicyltae gained 23.1 percent in the initial period on basal diet only, and required 4.2 days to lose 11.7 percent before the change in diet. At the end of 32 days they showed a weight of 119.7 percent above their peak weight. This is 5.7 percent above that of the first group.

Guinea pigs on basal diet only, Table IV, gained an average of 14.8 percent in 15.6 days in the initial period. They lost 9 percent of their peak weight in 5.4 days and survived an average of 17.2 days on the basal diet. At death they weighted 56.5 percent of their peak weight. The heavy animals on basal diet plus 1 percent salicylate, Table V, showed a slight gain in the initial period, but only a loss of 5.1 percent in 5 days on basal only. This group was seen to have a subsequent rise in weight not noted in light weight animals on the same diet. As was stated before in a preced-

ing paragraph this group was considered as not suffering a sufficient loss in weight after the peak was reached to warrant acceptance as required in the procedure. However, the heavy group survived, on salicylate, a period of 42 days with a death weight of 65.3 percent of their peak weight. The lighter animals, Table VI, showed an average gain of 11.3 percent in 16.5 days with a loss of 10.7 percent in 5.3 days before change in diet to 1 percent salicylate. After a survival period of 20 days, these animals showed a death weight of 62.1 percent. This is 5.6 percent above that of the scorbutic control group IV. Table VII, including all animals on basal diet and 1½ percent salicylate showed an average gain of 10.6 percent in 11.8 days on basal diet only, and required 4.8 days to lose 8.5 percent of their weight before treatment. In 15.7 days after the peak weight these animals showed an average death weight of 63.8 percent which is 7.3 percent above the average death weight of the control scorbutic animals.

Discussion

There was a large variation in the time required for each animal to reach their peak weight on basal diet without vitamin C. Table VIII shows that the time required by the various groups of animals varied from 11.3 days to 16 days on basal diet before such loss in weight occurred. This may possibly be explained in terms of the ability of individual animals to retain vitamin C.

The survival period of animals on scorbutic diet appeared to be influenced by the addition of sodium salicylate to the diet. It was noted that the animals receiving a 1 percent diet lived 3 days longer than those on scorbutic diet alone, while those that received the higher concentration died earlier. Thus it seems that lighter concentrations of salicylate may prolong life somewhat while the heavier tends to decrease it. This may possibly be attributed to a toxic effect of sodium salicylate on the scorbutic animal when given in the higher concentration.

The weights of animals also appeared to be directly influenced by the use of salicylate whether in the recovery animal or in the scorbutic animal. The average weight of both the recovery groups and the scorbutic groups which received salicylates were on an average higher than the average

weight of their respective control groups. This gain of 5 to 20 percent in the recovery group and of 6 to 7 percent in the scorbutic group with salicylate diet, over that of the controls may possibly be explained by the production of an edema in the animal or by an analgesic action of sodium salicylate which may permit the animal to feel better during the experimental period.

The use of salicylates to replace vitamin C seems to be contraindicated by the post-mortem findings which revealed that those animals which were fed sodium salicylate with basal diet had gross lesions of scurvy quite similar to those animals on scorbutic diet alone. This with the above noted results of salicylate on the survival period and death weight of guinea pigs in experimental scorbutus certainly indicates that salicylates could not replace vitamin C. Since salicylates may influence to a small degree the length of life and the death weight of such animals it again raises the question whether death in experimental scorbutus may be due to vitamin C deficiency or to starvation.

Conclusions

1. Sodium salicylate in concentrations of 1 percent and 1½ percent in the diet did not have any appreciable toxic effect upon guinea pigs receiving basal diet and vitamin C.

2. The length of time that animals showed a progressive increase in weight on scorbutic diet may possibly be explained in terms of the ability of individual animals to retain vitamin C.

3. Sodium salicylate was found to increase the weight of guinea pigs whether on recovery or in a state of experimental scorbutus.

4. The length of the survival period appears to be influenced by the concentration of sodium salicylate in the diet. A 1 percent concentration tends to increase the length of life while the 1½ percent concentration tends to have the opposite effect.

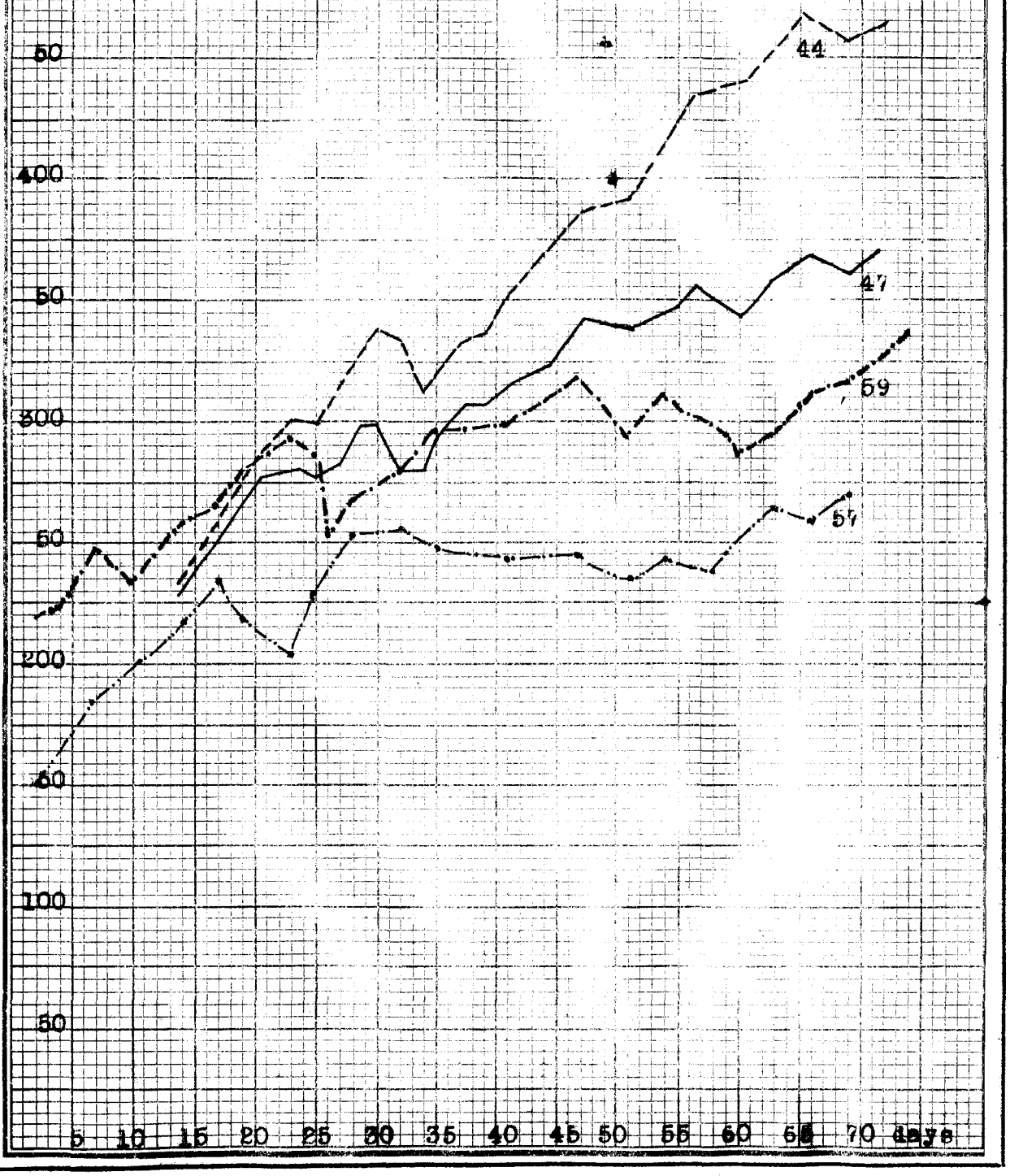
5. Sodium salicylate in concentrations of 1½ percent in the diet seems to exert an added toxic effect on the guinea pig in experimental scorbutus.

6. Sodium salicylate cannot be used to replace vitamin C in the treatment of experimental scorbutus.

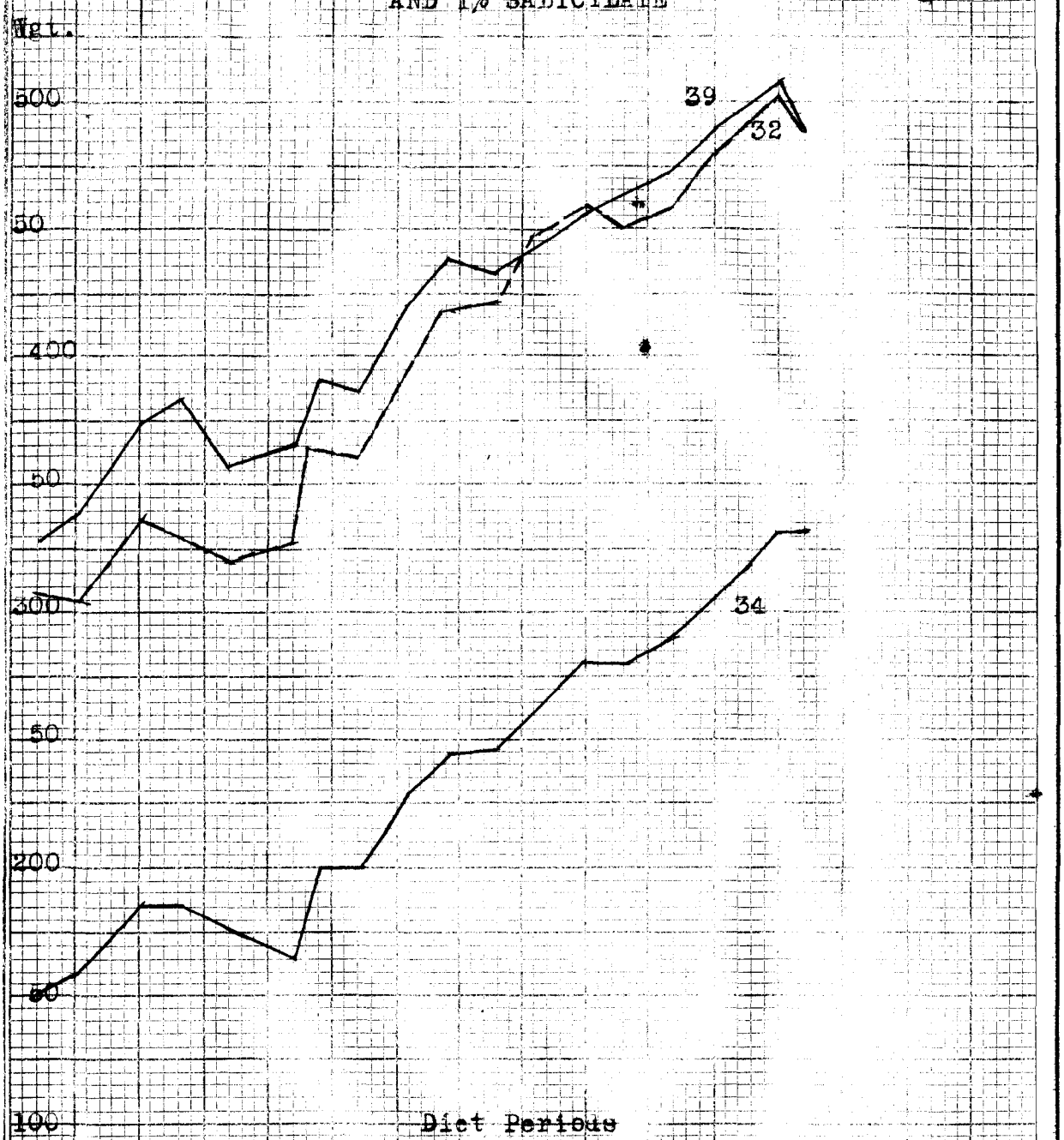
BASAL DIET PLUS CEVITAMIC ACID OR TOMATO JUICE

Diet Periods

Animal	Control	Basal	Experimental	Peak
44	to 13th.day	to 34th.day	to 77th.day	30th.
47	13	32	77	30th.
57	14	23	74	17th.
59	14	26	69	23rd.



BASAL DIET PLUS TOMATO JUICE AND 1% SALICYLATE



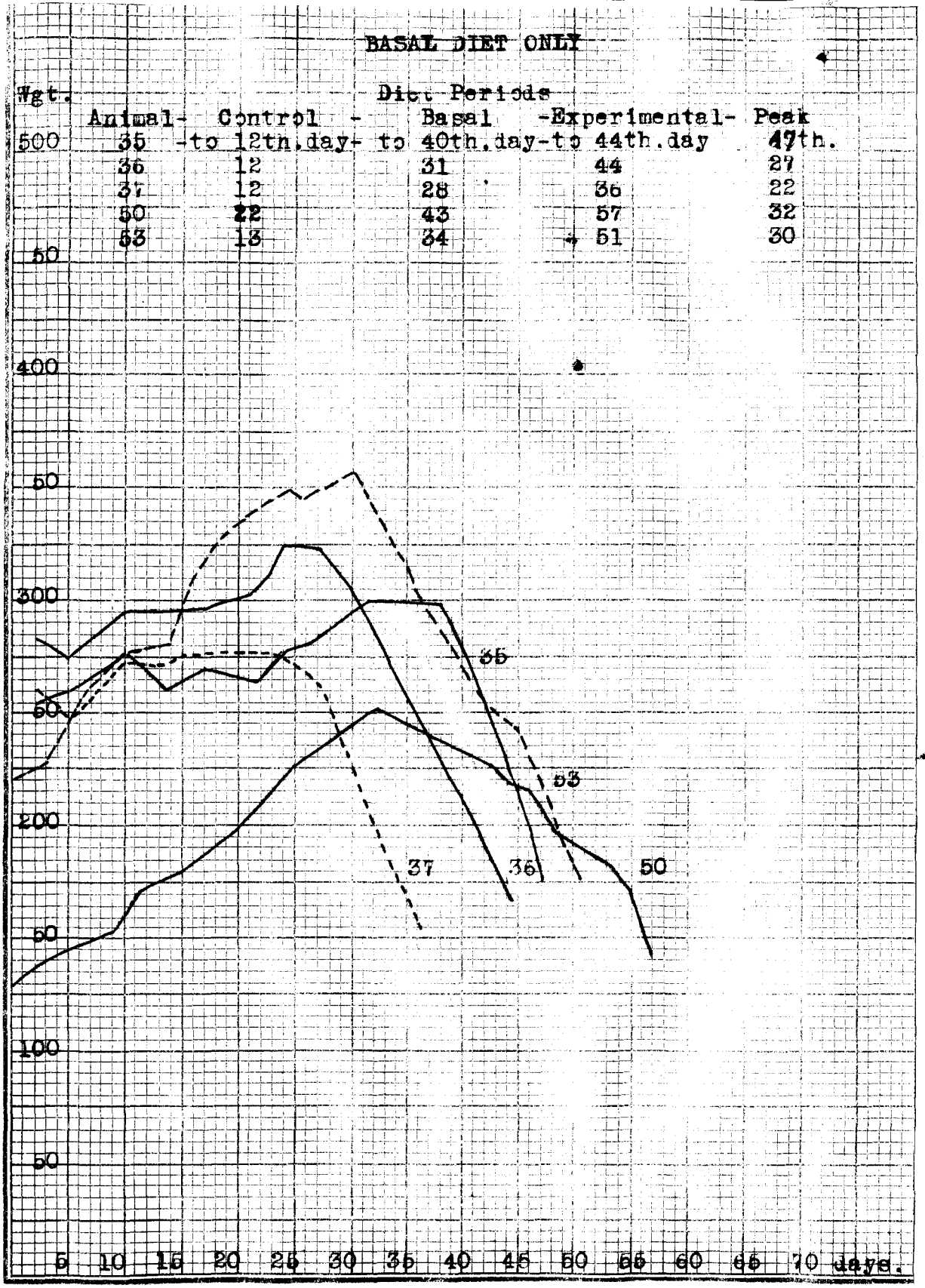
Diet Periods

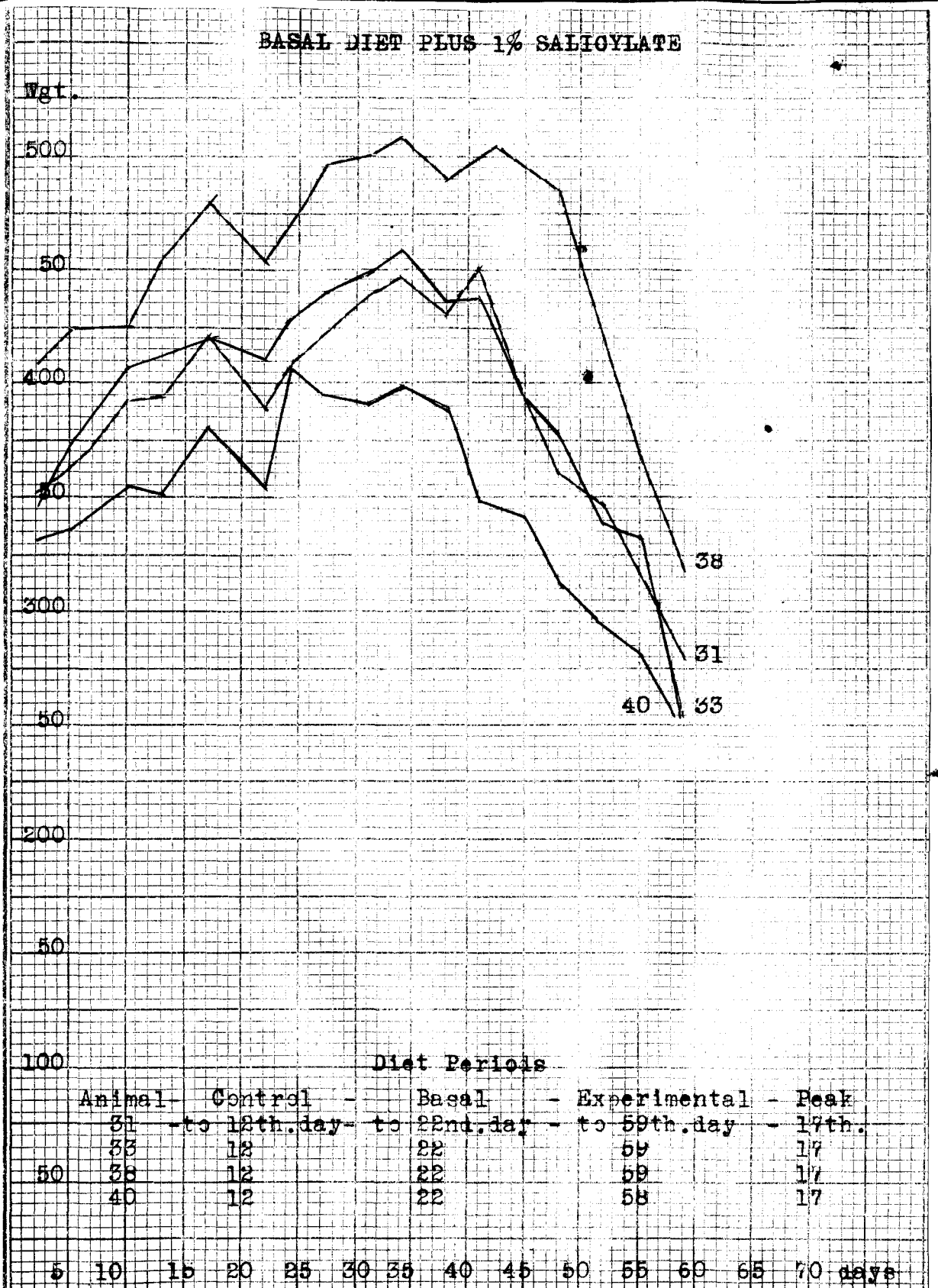
Animal	Control	Basal	Experimental	Peak
32	to 11th. day	to 19th. day	to 62nd. day	11th.
34	11	19	62	13
39	11	19	62	13

5 10 15 20 25 30 35 40 45 50 55 60 65 70 days.

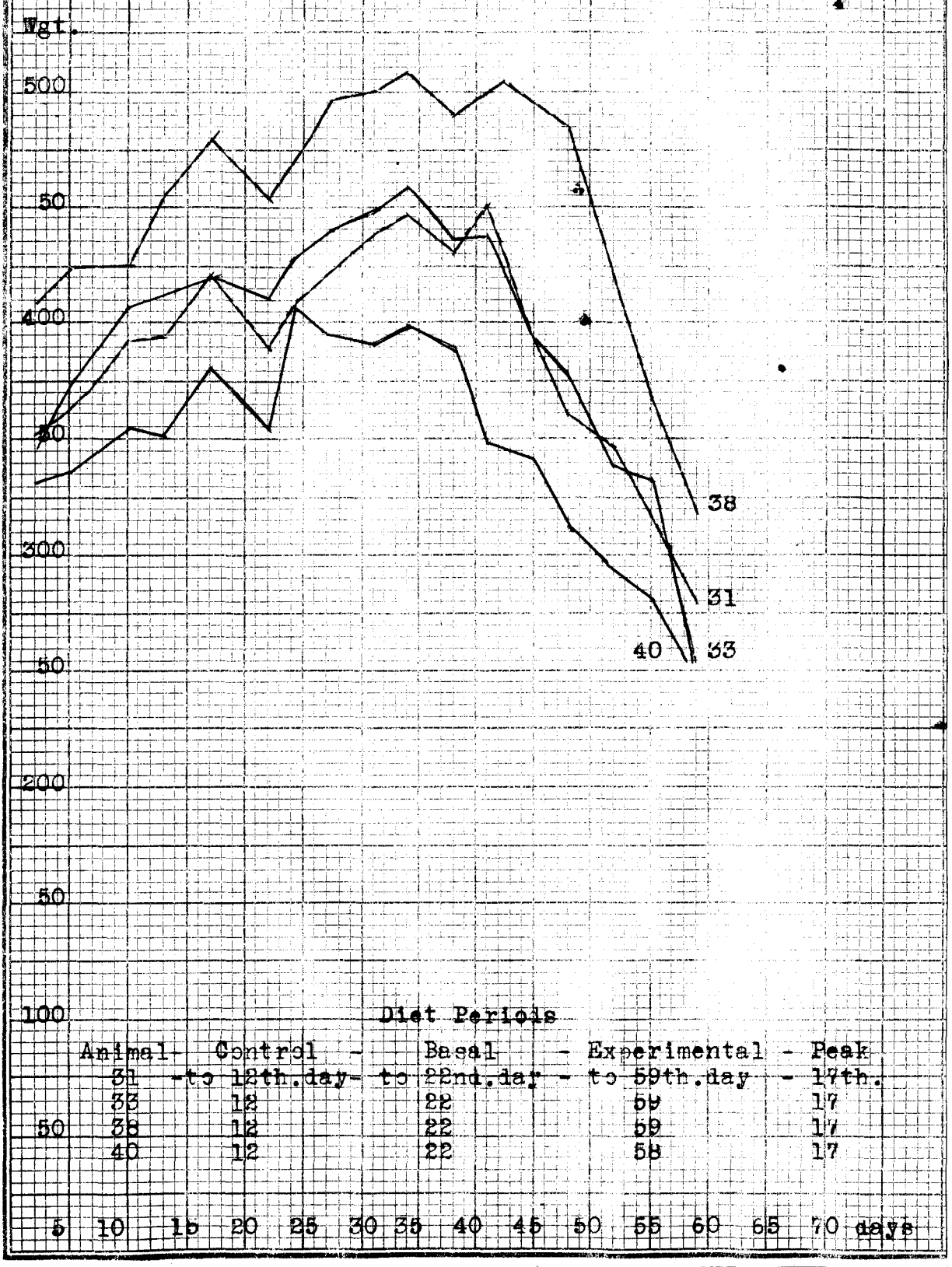
BASAL DIET ONLY

Wgt.	Animal	Control	Basal	Experimental	Peak
		Diet Periods			
	35	to 12th.day	to 40th.day	to 44th.day	47th.
	36	12	31	44	27
	37	12	28	36	22
	50	22	43	57	32
	53	13	34	51	30



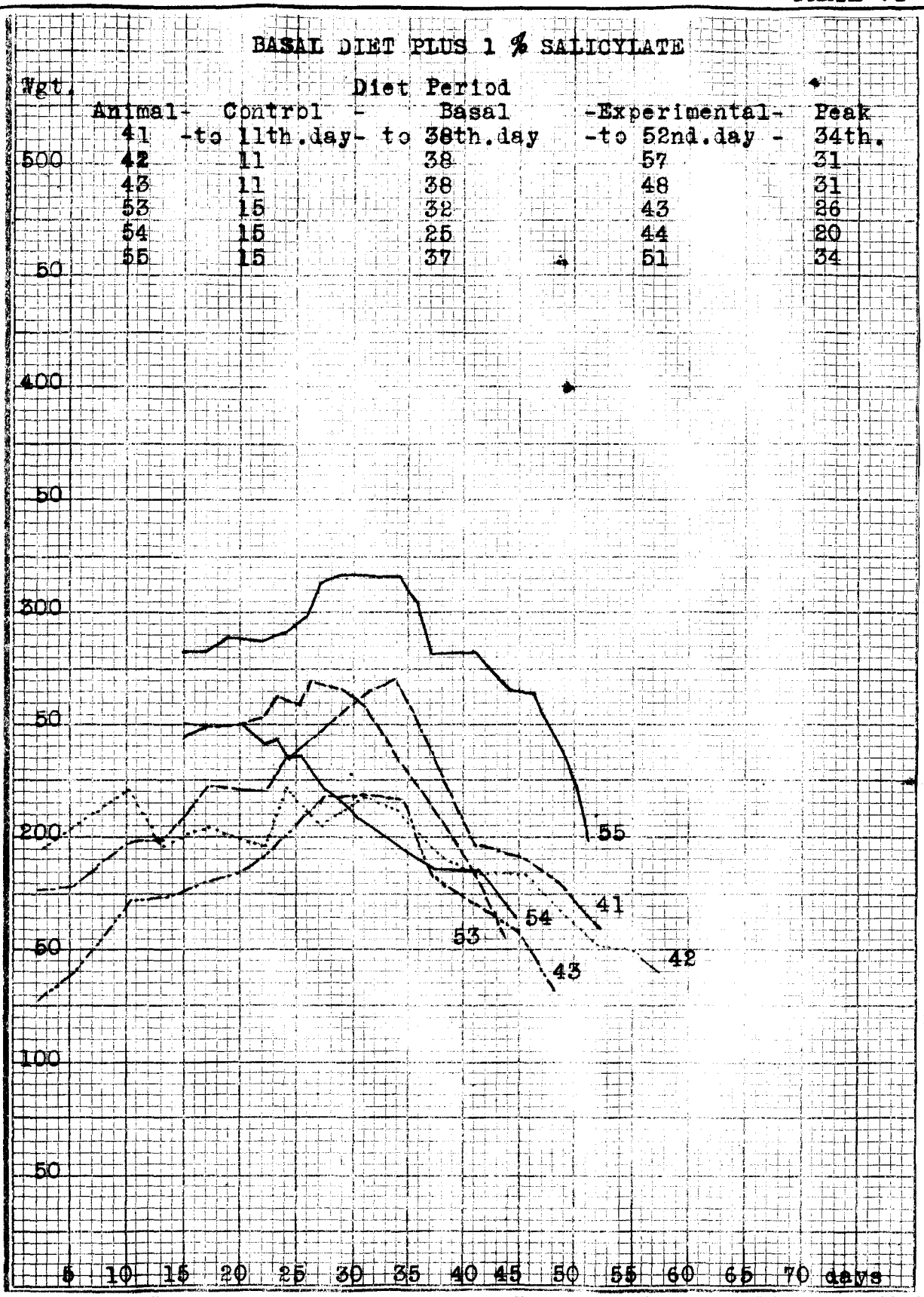

BASAL DIET PLUS 1% SALICYLATE
Wgt.
500
50
400
300
50
200
50
100
38
31
40
33
Diet Periods
Animal- Control - Basal - Experimental - Peak
31 -to 12th.day- to 22nd.day - to 59th.day - 17th.
33 12 22 59 17
38 12 22 59 17
40 12 22 58 17
5 10 15 20 25 30 35 40 45 50 55 60 65 70 days

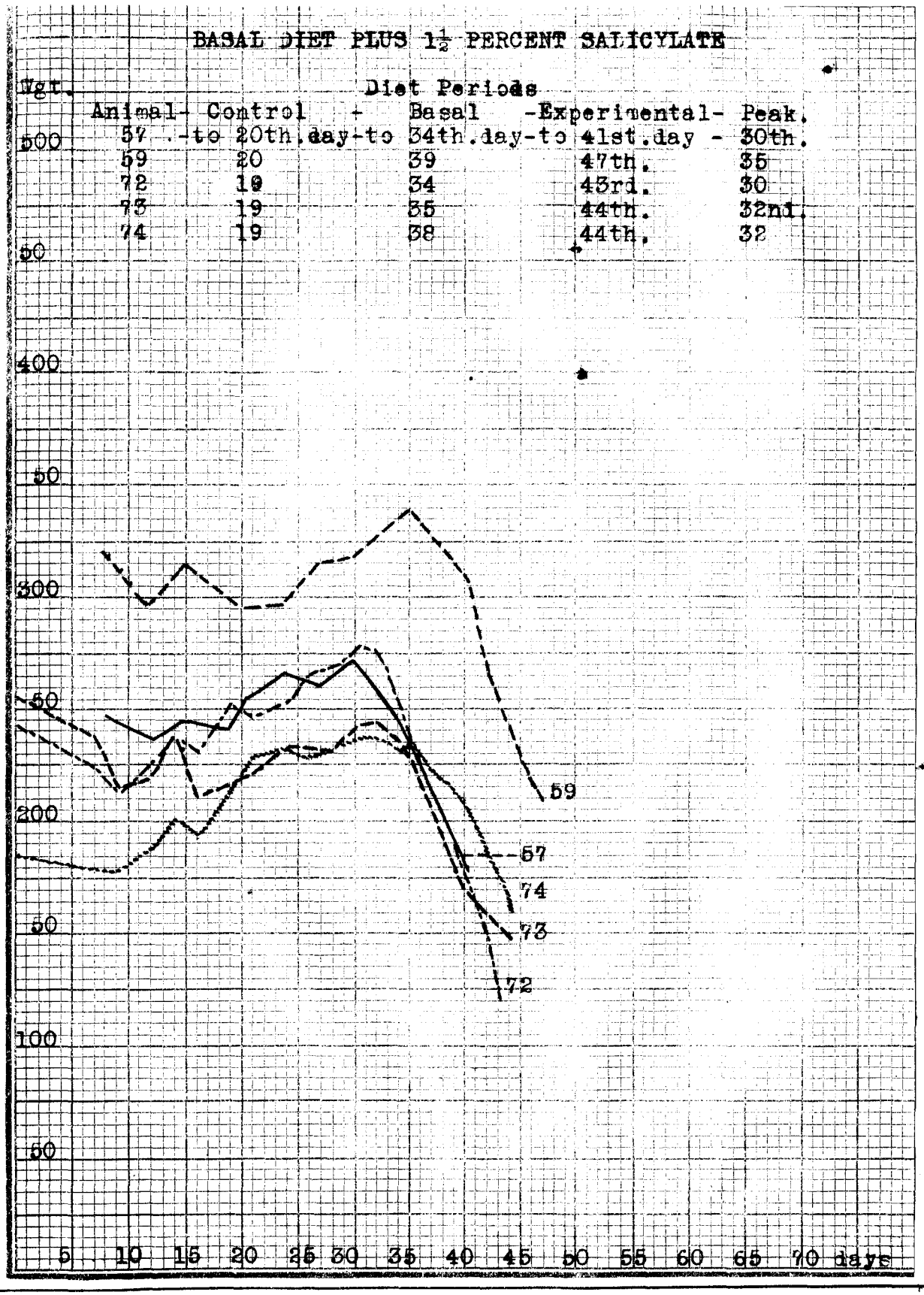
BASAL DIET PLUS 1% SALICYLATE



BASAL DIET PLUS 1 % SALICYLATE

Wgt.	Animal	Control Diet Period	Basal	Experimental	Peak
	41	to 11th.day	to 38th.day	to 52nd.day	34th.
500	42	11	38	57	31
	43	11	38	48	31
	53	15	32	43	26
	54	15	25	44	20
	55	15	37	51	34




BASAL DIET PLUS 1½ PERCENT SALICYLATE
Diet Periods
Animal- Control - Basal -Experimental- Peak.
57 .-to 20th.day-to 34th.day-to 41st.day - 30th.
59 20 39 47th. 35
72 19 34 43rd. 30
73 19 35 44th. 32nd.
74 19 38 44th. 32
Wgt.
500
400
300
200
100
59
57
74
73
72
5 10 15 20 25 30 35 40 45 50 55 60 65 70 days

BASAL DIET PLUS 1½ PERCENT SALICYLATE

Animal	Control Diet Period	Basal	Experimental	Peak
62	to 34th. day	to 48th. day	to 56th. day	34th.
63	34th.	49th.	55th.	45th.
64	20th.	38th.	48th.	36th.
86	20th.	38th.	56th.	36th.
76	20th.	46th.	59th.	46th.
90	22nd.	35th.	60th.	34th.
	22nd.	35th.	50th.	32nd.

5 10 15 20 25 30 35 40 45 50 55 60 65 70 days

BASAL DIET PLUS 1½ PERCENT SALICYLATE

Wgt.

Animal	Control	Basal	Experimental	Peak
	Diet Periods			
	to 44th. day	to 62nd. day	to 71st. day	59th.
68	44	60	66	44
67	25	49	58	44
69	25	34	44	30
70	25	46	56	45
71	25	45	57	43

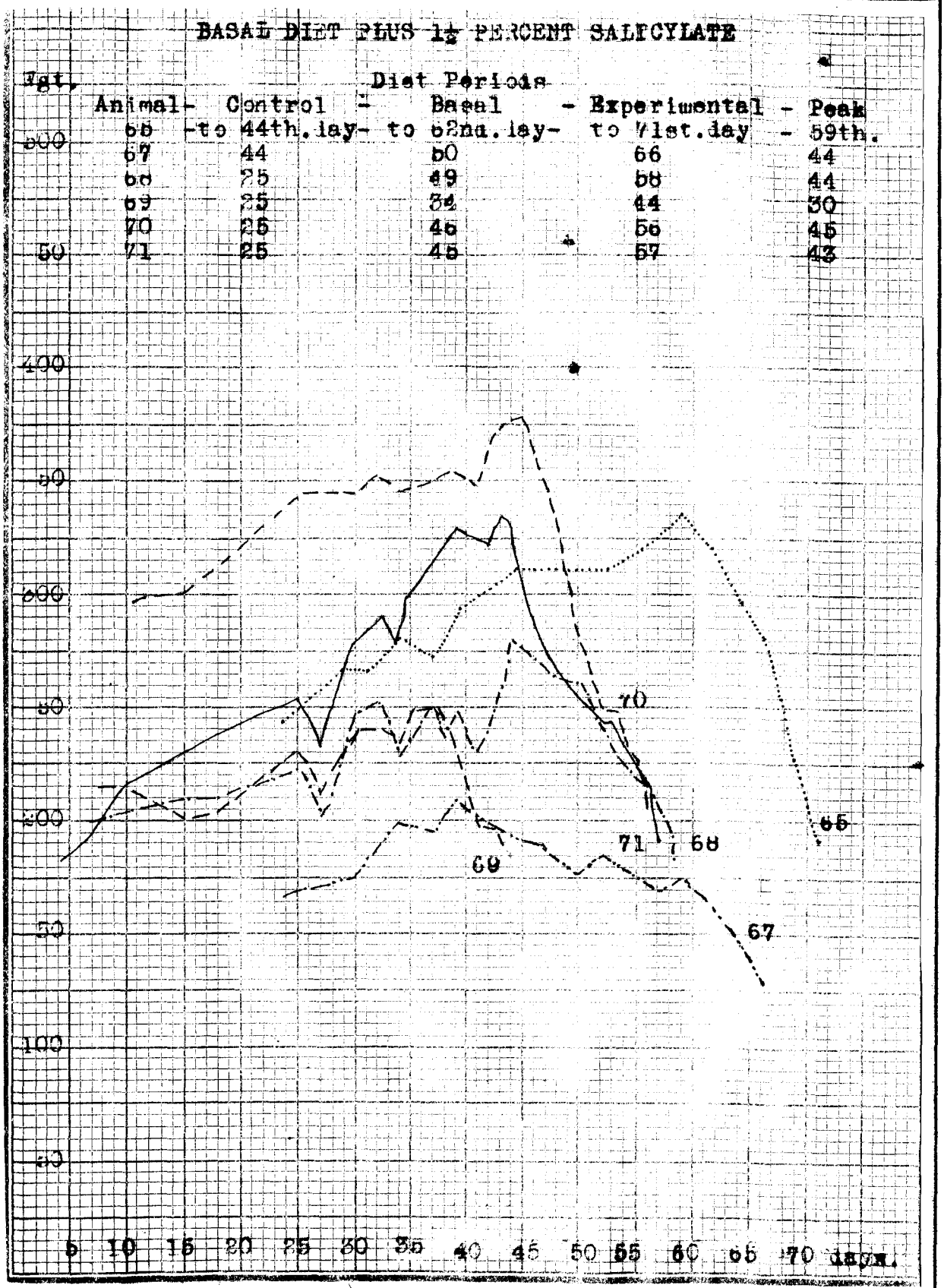


TABLE I

GUINEA PIGS ON BASAL DIET AND VITAMIN C

Number of animal	Initial Diet Period				Experimental diet Period	
	Days before loss occurred	Gain of weight	Days required to lose 10 %	Actual loss of weight in %	Time in days	Weight in %
44	17	31.4	4	8.3	32	132
47	17	24.8	2	7.0	32	118
57	3	7.2	6	12.8	32	101
59	9	11.2	3	12.6	32	105
Average	11.5	18.6	3.7	10.2	32	114

TABLE II

GUINEA PIGS ON BASAL DIET WITH VITAMIN C AND 1% SALICYLATE

Animal number	Initial Diet Period				Experimental Diet Period.	
	Days before loss occurred	Gain of weight	Days required to lose 10%	Actual loss of weight in %	Time in days	Weight in %
32	0	0.6	8	4.7	32	135.5
34	0	0	6	11.3	32	151
39	2	2	6	6.8	32	118.5
Average	1.3	0.6	6.6	7.6	32	134.6

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TABLE III

GUINEA PIGS ON BASAL DIET WITH VITAMIN C AND 1½% SALICYLATE

Number of animal	Initial Diet Period				Experimental Diet Period	
	Days before loss occurred	Gain of weight	Days required to lose 10%	Actual loss of weight in %	Time in days	Weight in %
78	16	19.5	9	9.3	32	120
79	9	25.5	2	11.0	32	134.5
82	16	20.5	3	13.8	32	93.3
81	16	26.8	3	13.0	32	130.8
Average	13.6	23.1	4.2	11.7	32	119.7

TABLE IV

GUINEA PIGS ON BASAL DIET ONLY

Number of animal	Initial Diet Period				Experimental Diet Period	
	Days before loss occurred	Gain of weight	Days required to lose 10 %	Actual loss of weight in %	Time in days	Weight in %
35	26	10.2	2	7.0	9	58.7
36	15	8.6	4	8.6	17	51.7
37	10	.2.0	6	9.7	14	56.0
50	10	32.0	11	10.7	25	56.4
53	17	21.5	4	9.0	21	49.5
Average	15.6	14.8	5.4	9.0	17.2	56.5

TABLE V

GUINEA PIGS ON BASAL DIET WITH 1% SALICYLATE

Number of animal	Initial Diet Period			Experimental Diet Period		
	Days before loss occurred	Gain of weight	Days required to lose 10 %	Actual loss of weight in %	Time in days	Weight in %
31	5	6.4	5	7.3	42	66.8
33	5	2.5	5	2.0	42	60.7
38	5	8.0	5	5.0	42	66.6
40	5	7.2	5	6.5	41	66.8
Average	5	6.0	5	5.1	42	65.3

TABLE VI

GUINEA PIGS ON BASAL DIET WITH ONE PERCENT SALICYLATE

Number of animal	Initial DIet Period				Experimental Diet Period	
	Days before loss occurred	Gain of weight	Days required to lose 10%	Actual loss of weight in %	Time in Days	Weight in %
41	23	19.2	4.0	8.2	18	59.7
42	20	3.2	7.0	12.5	26	64.5
43	20	21.2	7.0	15.2	17	60.5
53	11	7.5	6.0	7.5	18	58.5
54	5	4.0	5.0	10.0	24	66.0
55	19	13.0	3.0	10.7	17	63.3
Average	16.5	11.3	5.3	10.7	20	62.1

TABLE VII

GUINEA PIGS ON BASAL DIET WITH 1½% SALICYLATE

Number of animal	Initial Diet Period				Experimental Diet Period	
	Days before loss occurred	Gain of weight	Days required to lose 10 %	Actual loss of weight in %	Time indays	Weight in %
57	10	8.0	4	9.8	11	66.3
59	15	12.7	4	6.8	12	62.2
72	11	10.0	4	10.0	13	43.2
73	12	10.7	3	7.5	12	81.3
74	12	9.2	6	8.5	12	67.5
62	0	0.0	14	13.6	22	73.7
63	11	10.8	4	10.8	10	71.5
84	9	9.5	9	13.7	19	65.0
85	9	13.8	9	17.3	27	76.4
86	25	10.6	1	8.8	14	67.8
76	12	14.8	2	17.5	26	53.5
90	10	26.7	3	11.8	18	64.0
65	15	7.0	3	5.3	12	56.7
67	0	0.0	6	8.5	22	66.5
68	19	18.2	5	4.0	14	67.5
69	5	5.0	4	2.5	14	76.5
70	20	9.3	1	8.0	11	54.5
71	18	24.2	2	10.7	14	56.5
Average	11.8	10.6	4.8	8.5	15.7	63.8

TABLE VIII

AVERAGES OF ALL GROUPS

Number of group	Initial Diet Period				Experimental Diet Period	
	Days before loss	Gain of weight	Days required to lose 10%	Actual loss of weight in %	Time in days	Weight in %
I	11.5	18.6	3.7	10.2	32*	114.0
II	1.3	0.6	6.6	7.6	32*	134.6
III	13.6	23.0	4.2	11.7	32*	119.7
IV	15.6	14.8	5.4	9.0	17.2	56.5
V	5.0	6.0	5.0	5.1	42.0	65.3
VI	16.5	11.3	5.3	10.7	20.0	62.1
VII	11.8	10.6	4.8	8.5	15.7	63.8

* Arbitrary end of experimental period for the recovery animals.

Bibliography

1. Bessey, O.A., M.L. Menten, and C.G. King. Pathologic Changes in the Organs of Scorbutic Guinea Pigs. Proc. Soc. Exp. Biol. and Med., 31; 455.
2. Barnes, R.E., and E.M. Hume. Relative Anti-scorbutic Value of Fresh, Dried and Heated Cow's Milk. Biochem. J., 13: 306, 1919.
3. Findlay, G.M. A Note on Experimental Scurvy in the Rabbit and on the Affects of Antenatal Nutrition. J. Path. and Bact., 24: 454, 1931.
4. Drummond, J.C. Note on the Role of the Anti-scorbutic Factor in Nutrition. Biochem. J., 13: 77, 1919.
5. Gross, L., L. Loewe, and B. Eliasoph. Attempts to Reproduce Rheumatic Fever in Animals. J. Exp. Med., 50: 41, 1929.
6. Menkin, V., S.B. Wolbach, and M.F. Menkin. Formation of Intracellular Substance by the Administration of Ascorbic Acid (Vitamin C) in Experimental Scorbutus. Am. J. Path., 10: 569, 1934.
7. Parsons, H.T., and M.K. Hutton. Some Further Observations Concerning the Antiscorbutic Requirements of the Rat. J. Biochem., 59: 97, 1924.
8. Purr, A. The Influence of Vitamin C on Intracellular Enzyme Action. Biol. Chem. J., 27: 1703, 1933.

9. Rhinehart, J.F., C.L. Connor, and S.R. Mettier. Further Observations of Pathologic Simularity Beween Experimental Scurvy Combined With Infection and Rheumatic Fever. J. Exp. Med., 59: 97, 1934.

10. Rhinehart, J.F., and S. R. Mettier. The Joints in Experimental Scurvy and in Scurvy with Superimposed Infection with a Consideration of the Possible Relation of Scurvy to Rheumatic Fever. Am. J. Path., 9: 952, 1933.

11. Rhinehart, J.F., and S.R. Mettier. The Heart Valves and Muscles in Experimental Scurvy with Superimposed Infection (with Notes on the Similarity of the Lesions to Rheumatic Fever). Am. J. Path., 10: 61, 1934.

12. Sherman, H.C., V.K. LaMer, and H.L. Campbell. The Quantitative Determination of the Antiscorbutic Vitamin (Vitamin C). J. Am Chem. Soc., 44: 165, 1922.

13. Wolbach, S.B., and P.R. Howe. Intercellular Substances in Experimental Scorbutus. Arch. of Path., 1: 1, 1926.